



Consommation et
Affaires commerciales Canada
Bureau des brevets
Ottawa, Canada
K1A 0C9

Consumer and
Corporate Affairs Canada
Patent Office

(21) (A1) 2,105,306
(88) 1992/03/13
(43) 1992/09/16

6,005,4/05

(51) INTL. CL. ⁵ C12N-015/12; C12Q-001/68; C12N-005/10; C07K-015/28;
C07K-013/00; C07K-017/00; A61K-039/395; A61K-047/48;
A61K-043/00; A61K-037/02; G01N-033/574; G01N-033/566;
G01N-033/68

(19) (CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Receptors for Bombesin-Like Peptides

(72) Battey, James F., Jr. - U.S.A. ;
Corjay, Martha H. - U.S.A. ;
Fathi, Zahra - U.S.A. ;
Feldman, Richard I. - U.S.A. ;
Harkin, Richard N. - U.S.A. ;
Slattery, Timothy K. - U.S.A. ;
Wada, Etsuko - U.S.A. ;
Wu, James M. - U.S.A. ;

(71) Berlex Laboratories, Inc. - U.S.A. ;
Government of the United States of America as
represented by The Secretary of the Department of Health
and Human Services (The) - U.S.A. ;

(30) (US) 670,603 1991/03/15
(US) 771,332 1991/10/03

(57) 86 Claims

Notice: This application is as filed and may therefore contain an
incomplete specification.

Canada

CCA 3254 (10-92) 41 7530-21-936-3254

BEST AVAILABLE COPY

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12N 15/12, 5/10, A61K 37/02 G01N 33/68, 33/577, A61K 39/395 C07K 13/00, C12B 21/00 C12Q 1/68, G01N 33/574, 33/543 C12N 15/86		A2	(11) International Publication Number: WO 92/16623
2105306			(43) International Publication Date: 1 October 1992 (01.10.92)
(21) International Application Number: PCT/US92/02091		(74) Agents: DOW, Karen, B. et al.; Townsend and Townsend, One Market Plaza, 2000 Steuart Tower, San Francisco, CA 94105 (US).	
(22) International Filing Date: 13 March 1992 (13.03.92)		(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), NO, SE (European patent).	
(30) Priority data: 670,603 15 March 1991 (15.03.91) US 771,332 3 October 1991 (03.10.91) US		Published <i>Without international search report and to be republished upon receipt of that report.</i>	
(71) Applicants: BERLEX LABORATORIES, INC. [US/US]; 110 East Hanover Avenue, Cedar Knolls, NJ 07927 (US). THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Patent Branch, Office of Technology Transfer, Box OTT, Bethesda, MD 20892 (US).			
(72) Inventors: BATTEY, James, F., Jr. ; 13 Savannah Court, Bethesda, MD 20817 (US). CORJAY, Martha, H. ; 1106 Broken Avenue, Wilmington, DE 19808 (US). FATHI, Zahra ; 13811-43 Castle Boulevard, Silver Springs, MD 20904 (US). FELDMAN, Richard, I. ; 100 Pomona Avenue, El Cerrito, CA 94530 (US). HARKIN, Richard, N. ; 2819 Sea View Parkway, Alameda, CA 94501 (US). SLATTERY, Timothy, K. ; 2533 Claire Court, Mountain View, CA 94403 (US). WADA, Etsuko ; 12501 Village Square Terrace, Rockville, MD 20852 (US). WU, James, M. ; 5514 Zara Avenue, El Cerrito, CA 94530 (US).			

(54) Title: RECEPTORS FOR BOMBESIN-LIKE PEPTIDES**(57) Abstract**

Receptors for bombesin-like peptides are solubilized and purified in active form. The amino acid sequence and DNA encoding various subtypes of the receptors are disclosed. Uses of the purified receptor gene and polypeptide are disclosed, including means for screening for agonists and antagonists of the receptor ligands, for producing diagnostic or therapeutic reagents, and for producing antibodies. Therapeutic or diagnostic reagents and kits are also provided.

WHAT IS CLAIMED IS:

1. A DNA segment coding for a polypeptide having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.

5 2. The DNA segment according to Claim 1, wherein said DNA segment has the sequence shown in SEQ ID NO: 7, allelic or species variation thereof, or a unique portion thereof.

10 3. The DNA segment according to Claim 1, wherein said DNA segment encodes the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

15 4. A polypeptide free of proteins with which it is naturally associated and having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.

20 5. A polypeptide bound to a solid support and having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.

25 6. The polypeptide according to Claim 4 or 5, wherein said polypeptide has the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

30 7. A recombinant DNA molecule comprising a vector and the DNA segment according to Claim 1.

8. A cell that contains the recombinant DNA molecule according to Claim 7.

35 9. A method of producing a polypeptide having an amino acid sequence corresponding to human gastrin releasing peptide-receptor comprising culturing the cell according to Claim 8.

under conditions such that said DNA segment is expressed and said polypeptide thereby produced, and isolating said polypeptide.

5 10. An antibody having binding affinity to a recombinant human gastrin releasing peptide-receptor, or unique portions thereof.

10 11. The antibody according to Claim 10, wherein said receptor has the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

12. A DNA segment coding for a polypeptide having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

15 13. The DNA segment according to Claim 12, wherein said DNA segment has the sequence shown in SEQ ID NO:5, allelic or species variation thereof, or a unique portion thereof.

20 14. The DNA segment according to Claim 12, wherein said DNA segment encodes the amino acid sequence set forth in SEQ ID NO: 6, allelic or species variation thereof, or a unique portion thereof.

25 15. A polypeptide free of proteins with which it is naturally associated and having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

30 16. A polypeptide bound to a solid support and having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

35 17. The polypeptide according to Claim 15 or 16, wherein said polypeptide has the amino acid sequence set forth in SEQ ID NO: 6, allelic or species variation thereof, or a unique portion thereof.

18. A recombinant DNA molecule comprising a vector and the DNA segment according to Claim 12.

5 19. A cell that contains the recombinant DNA molecule according to Claim 18.

10 20. A method of producing a polypeptide having an amino acid sequence corresponding to neuromedin-B-preferring bombesin receptor comprising culturing the cell according to Claim 19 under conditions such that said DNA segment is expressed and said polypeptide thereby produced, and isolating said polypeptide.

15 21. An antibody having binding affinity to a recombinant neuromedin-B-preferring bombesin receptor, or unique portions thereof.

20 22. The antibody according to Claim 21, wherein said receptor has the amino acid sequence set forth in SEQ ID NO: 6, allelic or species variation thereof, or a unique portion thereof.

25 23. A recombinant or substantially pure nucleic acid comprising a sequence exhibiting substantial homology to a nucleotide sequence encoding a receptor, or a fragment thereof, for a bombesin-like peptide.

24. A nucleic acid of Claim 23 further comprising sequence encoding a second polypeptide, or fragment thereof.

30 25. A vector, cell, or organism comprising a nucleic acid of Claim 23.

35 26. A recombinant or substantially pure polypeptide comprising a region exhibiting substantial identity to an amino acid fragment of a receptor for a bombesin-like peptide.

27. A polypeptide of Claim 26 comprising a fragment of a second polypeptide.

5 28. A subcellular structure, cell, or organism comprising a protein of Claim 26.

10 29. A method of producing a receptor, or fragment thereof, for a bombesin-like peptide comprising expressing a nucleic acid of Claim 23.

15 30. A method of screening for a compound having binding affinity to a receptor for a bombesin-like peptide comprising the steps of:

a) producing said receptor by a method of Claim 29, and
b) assaying for the binding of said compound to said receptor.

20 31. An antibody having binding affinity for a receptor for a bombesin-like peptide or fragment thereof.

25 32. A method of simultaneously modulating a biological activity of a plurality of subtypes of receptors for bombesin-like peptides, comprising contacting said receptors with a compound which modulates said activity upon contacting said receptors.

30 33. An antibody exhibiting specificity of binding to at least one receptor for a bombesin-like peptide selected from the group consisting of:

a) a mouse R1BP, or fragment thereof;
b) a human R1BP, or fragment thereof;
c) a rat R2BP, or fragment thereof;
d) a human R2BP, or fragment thereof; and
e) a human R3BP, or fragment thereof.

34. A method of modulating biological activity of a receptor for a bombesin-like peptide comprising contacting said receptor with a composition selected from the group consisting of:

- 5 a) an antibody which binds to said receptor;
- b) a known agonist or antagonist to a receptor for a non-GRP bombesin-like peptide; and
- c) a ligand binding fragment from a receptor for a bombesin-like peptide.

10 35. A method of treating a host having cancer or exhibiting abnormal expression of a receptor for a bombesin-like peptide, comprising administering to said host a therapeutically effective amount of a composition comprising:

- 15 a) an antibody which binds to a receptor for a bombesin-like peptide;
- b) an agonist or antagonist to a receptor for a non-GRP bombesin-like peptide; or
- c) a ligand binding receptor, or fragment thereof, for a bombesin-like peptide.

20 36. A method of diagnosing for cancer in a host organism, comprising the steps of:

- a) contacting a sample from said host with a specific binding reagent to:
 - 25 i) a gene encoding a receptor for a bombesin-like peptide; or
 - ii) a receptor for a bombesin-like peptide; and
- b) measuring the level of binding of said reagent to said sample.

30 37. A method of evaluating binding affinity of a test compound to a receptor for a bombesin-like peptide, said method comprising the steps of:

- 35 a) contacting a sample containing said receptor with
 - i) a labeled compound having a known affinity for said receptor; and
 - ii) said test compound; and

157

b) measuring the level of bound labeled compound, said amount being inversely proportional to the amount of test compound which bound to said receptor.

5 38. A kit for determining the amount of a receptor for a bombesin-like peptide in a sample, comprising a compartment with a labeled compound having a known binding affinity for said receptor.

10 39. A kit for assaying antibody against a receptor for a bombesin-like peptide in a sample, comprising compartments having a said receptor and an antibody detection means.

15 40. A compound known to modulate activity of a receptor for a bombesin-like peptide, selected by a method of:

- a) contacting said compound with isolated or recombinant receptor, or fragment thereof, for a bombesin-like peptide; and
- b) evaluating the effect on biological activity by said contacting.

20 41. Isolated DNA encoding the gastrin releasing peptide receptor or fragment thereof encoding a biologically active gastrin releasing peptide receptor polypeptide.

25 42. Isolated DNA which encodes a biologically active protein having gastrin releasing peptide receptor activity and which is capable of hybridizing with the DNA of SEQ ID NO: 1.

30 43. The DNA of Claim 42 wherein said protein has the amino acid sequence of SEQ ID NO: 2.

35 44. Isolated DNA encoding proteins which are homologous to the gastrin releasing peptide receptor, and said DNA being isolated using gastrin releasing peptide receptor cDNA as a probe.

45. The DNA sequence according to Claims 41, 42, or 44 characterized in that it further comprises the respective regulatory sequences in the 5' and 3' flanks.

5 46. A DNA sequence hybridizing to a DNA sequence according to Claims 41, 42, or 44 and containing mutations selected from the group consisting of nucleotide substitutions, nucleotide deletions, nucleotide insertions and inversions of nucleotide stretches and coding for a protein having gastrin releasing peptide receptor activity.

10 47. A recombinant DNA molecule characterized in that it comprises a DNA sequence according to Claims 41, 42, or 44.

15 48. A recombinant DNA molecule characterized in that it comprises a DNA sequence according to Claims 41, 42, or 44 that is operably linked to a genetic control element.

20 49. The recombinant DNA molecule of Claim 48, characterized in that said control element is selected from the group consisting of procaryotic promoter systems and eucaryotic expression control systems.

25 50. The recombinant molecule of Claim 47 wherein said molecule is an expression vector for expressing eucaryotic cDNA coding for the gastrin releasing peptide receptor in a procaryotic or eucaryotic host, said vector being compatible with said host and wherein the eucaryotic cDNA coding for the gastrin releasing peptide receptor is inserted into said vector such that growth of the host containing said vector expresses said cDNA.

30 51. A host characterized in that the recombinant DNA molecule according to Claim 47 has been introduced into said host, and which expresses the protein encoded by said DNA.

52. The host of Claim 51 which is selected from the group consisting of: procaryotes including gram negative and gram positive organisms including *E. coli*; lower eucaryotes including yeasts; and higher eucaryotes including animal cells and mammalian cells including human.

53. A recombinant protein which is encoded by a DNA sequence according to Claim 47 and which is substantially free of protein or cellular contaminants, other than those derived from the recombinant host.

10 54. A pharmaceutical composition comprising the recombinant protein of Claim 53 and a conventional pharmaceutically acceptable carrier and/or diluent.

15 55. A vector comprising DNA encoding the gastrin releasing peptide receptor or a fragment thereof encoding a biologically active gastrin releasing peptide receptor polypeptide.

20 56. The vector of Claim 55 wherein said DNA is under the control of a viral promoter.

57. The vector of Claim 55 which further comprises DNA encoding a selection marker.

25 58. The vector of Claim 55 wherein said DNA encodes a predetermined, site-specific mutant gastrin releasing peptide receptor which has greater than about 50% amino acid homology with the gastrin releasing peptide receptor of SEQ ID NO: 2 and which exhibits biological activity in common with the gastrin releasing peptide receptor of SEQ ID NO: 2.

30 59. A cell from a multicellular organism transformed with the vector of Claim 55.

35 60. The cell of Claim 59 which is a mammalian cell.



160

61. A method comprising culturing the cell of Claim 59 in a nutrient medium, permitting the receptor to accumulate in the culture and recovering the receptor from the culture.

5 62. The method of Claim 61 wherein the receptor is recovered from the culture medium.

10 63. Antibodies having binding affinity to the recombinant gastrin releasing peptide receptor, or fragments thereof.

64. The antibodies of Claim 63 which are raised against the gastrin releasing peptide receptor, or fragments thereof.

15 65. The antibodies of Claims 63 or 64 wherein said receptor has the amino acid sequence of SEQ ID NO: 2.

20 66. The antibodies of Claim 65 wherein said fragments are selected from the group consisting of the following partial amino acid sequences: residues 1-39, inclusive; residues 64-77, inclusive; residues 98-115, inclusive; residues 138-157, inclusive; residues 176-209, inclusive; residues 236-266, inclusive; residues 288-300, inclusive; and residues 330-385, inclusive.

25 67. The antibodies of Claim 63 which are non-neutralizing antibodies.

68. The antibodies of Claim 63 which are neutralizing antibodies.

30 69. The antibodies of Claim 63 which are conjugated to toxins.

70. The antibodies of Claim 63 which are conjugated to radionuclides.

35 71. A kit for determining the concentration of gastrin releasing peptide receptor in a sample comprising a labeled

compound having known binding affinity for the gastrin releasing peptide receptor, recombinant gastrin releasing peptide receptor, and a means for separating bound from free labeled compound.

5

72. The kit of Claim 71 wherein said means for separating is a solid phase for immobilizing the gastrin releasing peptide receptor.

10

73. The kit of Claim 71 wherein said labeled compound is a ligand.

74. The kit of Claim 73 wherein said ligand is gastrin releasing peptide.

15

75. The kit of Claim 71 wherein said labeled ligand is an antibody.

20

76. The kit of Claim 72 wherein said solid phase contains a capture molecule.

77. The kit of Claim 76 wherein said capture molecule is an antibody to the gastrin releasing peptide receptor.

25

78. A kit for determining the binding affinity of a test compound to the gastrin releasing peptide receptor comprising a test compound, a labeled compound having known binding affinity for the gastrin releasing peptide receptor, recombinant gastrin releasing peptide receptor and a means for separating bound from free labeled compound.

30

79. The kit of Claim 78 wherein said means for separating is a solid phase for immobilizing the solubilized gastrin releasing peptide receptor.

35

80. The kit of Claim 78 wherein said labeled compound is a ligand.

162

81. The kit of Claim 80 wherein said ligand is gastrin releasing peptide.

5 82. The kit of Claim 78 wherein said labeled ligand is an antibody.

83. The kit of Claim 79 wherein said solid phase contains a capture molecule.

10 84. The kit of Claim 83 wherein said capture molecule is an antibody to the gastrin releasing peptide receptor.

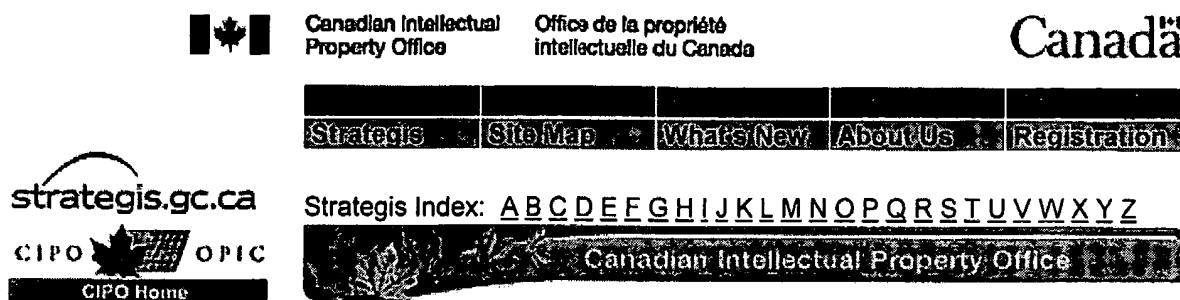
15 85. A method of treating patients having a disease or disorder associated with abnormal expression or abnormal triggering of the gastrin releasing peptide receptor comprising administering antibodies having binding affinity to the recombinant gastrin releasing peptide receptor.

20 86. A method of treating patients having a disease or disorder associated with abnormal expression or abnormal triggering of the gastrin releasing peptide receptor comprising administering recombinant gastrin releasing peptide receptor, or fragments thereof.

25

30

35



Canadian Patents Database

(12) Patent Application:

(11) CA 2105306

(54) RECEPTORS FOR BOMBESIN-LIKE PEPTIDES

(54) RECEPTEURS POUR PEPTIDES ASSIMILES A LA BOMBESINE

[View or Download Images](#)

ABSTRACT:

2105306 9216623 PCTABS00016 Receptors for bombesin-like peptides are solubilized and purified in active form. The amino acid sequence and DNA encoding various subtypes of the receptors are disclosed. Uses of the purified receptor gene and polypeptide are disclosed, including means for screening for agonists and antagonists of the receptor ligands, for producing diagnostic or therapeutic reagents, and for producing antibodies. Therapeutic or diagnostic reagents and kits are also provided.

CLAIMS: [Show all claims](#)

*** Note: Data on abstracts and claims is shown in the official language in which it was submitted.

(72) Inventors (Country):

BATTEY, JAMES F., JR. (United States)
CORJAY, MARTHA H. (United States)
FATHI, ZAHRA (United States)
FELDMAN, RICHARD I. (United States)
HARKIN, RICHARD N. (United States)
SLATTERY, TIMOTHY K. (United States)
WADA, ETSUKO (United States)
WU, JAMES M. (United States)

(73) Owners (Country):

GOVERNMENT OF THE UNITED STATES OF AMERICA
AS REPRESENTED BY THE (United States)
GOVERNMENT OF THE UNITED STATES OF AMERICA
AS REPRESENTED BY THE (United States)

(71) Applicants (Country):

(74) Agent: BORDEN LADNER GERVAIS LLP

(45) Issued:

(22) Filed: Mar. 13, 1992

(43) Laid Open: Sep. 16, 1992

Examination requested: July 4, 1994

(51) International Class (IPC):

A61K 43/00
 C07K 17/00
 C12N 5/10
 C12N 15/12
 A61K 38/22
 C07K 16/28
 A61K 39/395
 A61K 47/48
 G01N 33/566
 G01N 33/574
 G01N 33/68
 C12Q 1/68
 C07K 14/72

Patent Cooperation Treaty (PCT): Yes

(85) <u>National Entry:</u>	Aug. 31, 1993
(86) <u>PCT Filing number:</u>	PCT/US1992/002091
(87) <u>International publication number:</u>	WO1992/016623

(30) Application priority data:

Application No.	Country	Date
670,603	United States	Mar. 15, 1991
771,332	United States	Oct. 3, 1991

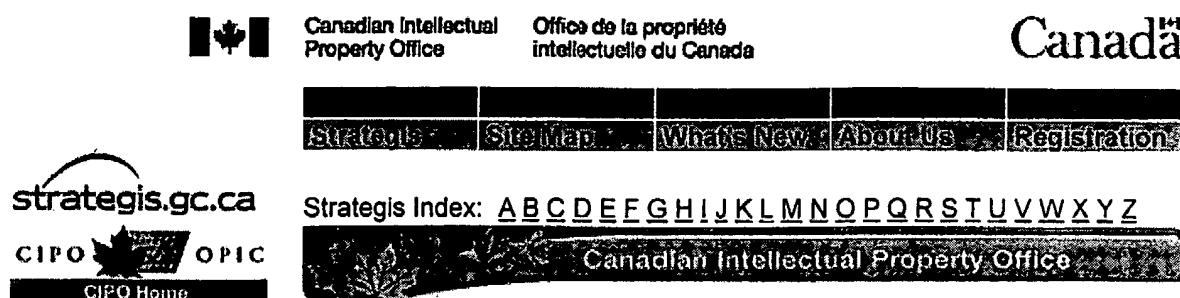
Availability of licence: N/A

Language of filing: English

View or Download Images :

- Cover Page Image
- Abstract Image
- Claims Image
- Disclosures Image
- Drawings Image
- Representative Drawing Image

 [View the Image](#)
 [Download in Adobe PDF](#)



Canadian Patents Database

Patent Document Number 2105306 :
RECEPTORS FOR BOMBESIN-LIKE PEPTIDES

RECEPTEURS POUR PEPTIDES ASSIMILES A LA BOMBESINE

CLAIMS:

WO 92/16623 PCT/US92/02091

152

WHAT IS CLAIMED IS:

1. A DNA segment coding for a polypeptide having an amino acid sequence corresponding to a human gastrin releasing peptidereceptor, or a unique portion thereof.
2. The DNA segment according to Claim 1, wherein said DNA segment has the sequence shown in SEQ ID NO: 7, allelic or species variation thereof, or a unique portion thereof.
3. The DNA segment according to Claim 1, wherein said DNA segment encodes the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.
4. A polypeptide free of proteins with which it is naturally associated and having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.
5. A polypeptide bound to a solid support and having an amino acid sequence corresponding to a human gastrin releasing peptide-receptor, or a unique portion thereof.
6. The polypeptide according to Claim 4 or 5, wherein said polypeptide has the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

7. A recombinant DNA molecule comprising a vector and the DNA segment according to Claim 1.

8. A cell that contains the recombinant DNA molecule according to Claim 7.

9. A method of producing a polypeptide having an amino acid sequence corresponding to human gastrin releasing peptidereceptor comprising culturing the cell according to Claim 8

WO 92/16623 PCT/US92/02091

153 under conditions such that said DNA segment is expressed and said polypeptide thereby produced, and isolating said polypeptide.

10. An antibody having binding affinity to a recombinant human gastrin releasing peptide-receptor, or unique portions thereof.

11. The antibody according to Claim 10, wherein said receptor has the amino acid sequence set forth in SEQ ID NO: 8, allelic or species variation thereof, or a unique portion thereof.

12. A DNA segment coding for a polypeptide having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

13. The DNA segment according to Claim 12, wherein said DNA segment has the sequence shown in SEQ ID NO:5, allelic or species variation thereof, or a unique portion thereof.

14. The DNA segment according to Claim 12, wherein said DNA segment encodes the amino acid sequence set forth in SEQ ID NO:

6, allelic or species variation thereof, or a unique portion thereof.

15. A polypeptide free of proteins with which it is naturally associated and having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

16. A polypeptide bound to a solid support and having an amino acid sequence corresponding to a neuromedin-B-preferring bombesin receptor, or a unique portion thereof.

17. The polypeptide according to Claim 15 or 16, wherein said polypeptide has the amino acid sequence set forth in SEQ ID NO:

6, allelic or species variation thereof, or a unique portion thereof.

WO 92/16623 PCT/US92/02091

154

18. A recombinant DNA molecule comprising a vector and the DNA segment according to Claim 12.

19. A cell that contains the recombinant DNA molecule according to Claim 18.

20. A method of producing a polypeptide having an amino acid sequence corresponding to neuromedin-B-preferring bombesin receptor comprising culturing the cell according to Claim 19 under conditions such that said DNA segment is expressed and said polypeptide thereby produced, and isolating said polypeptide.
21. An antibody having binding affinity to a recombinant neuromedin-B-preferring bombesin receptor, or unique portions thereof.
22. The antibody according to Claim 21, wherein said receptor has the amino acid sequence set forth in SEQ ID NO: 6, allelic or species variation thereof, or a unique portion thereof.
23. A recombinant or substantially pure nucleic acid comprising, a sequence exhibiting substantial homology to a nucleotide sequence encoding a receptor, or a fragment thereof, for a bombesin-like peptide.
24. A nucleic acid of Claim 23 further comprising sequence encoding a second polypeptide, or fragment thereof.

25. A vector, cell, or organism comprising a nucleic acid of Claim 23.

26. A recombinant or substantially pure polypeptide comprising a region exhibiting substantial identity to an amino acid fragment of a receptor for a bombesin-like peptide.

WO 92/16623 PCT/US92/02091

155

27. A polypeptide of Claim 26 comprising a fragment of a second polypeptide.

28. A subcellular structure, cell, or organism comprising a protein of Claim 26.

29. A method of producing a receptor, or fragment thereof, for a bombesin-like peptide comprising expressing a nucleic acid of Claim 23.

30. A method of screening for a compound having binding affinity to a receptor for a bombesin-like peptide comprising the steps of:

- a) producing said receptor by a method of Claim 29, and
- b) assaying for the binding of said compound to said receptor.

31. An antibody having binding affinity for a receptor for a bombesin-like peptide or fragment thereof.

32. A method of simultaneously modulating a biological activity of a plurality of subtypes of receptors for bombesinlike peptides, comprising contacting said receptors with a compound which modulates said activity upon contacting said receptors.

33. An antibody exhibiting specificity of binding to at least one receptor for a bombesin-like peptide selected from the group consisting of:

- a) a mouse R1BP, or fragment thereof;
- b) a human R1BP, or fragment thereof;
- c) a rat R2BP, or fragment thereof;
- d) a human R2BP, or fragment thereof; and

e) a human R3BP, or fragment thereof.
WO 92/16623 PCT/US92/02091

156

34. A method of modulating biological activity of a receptor for a bombesin-like peptide comprising contacting said receptor with a composition selected from the group consisting of:

- a) an antibody which binds to said receptor;
- b) a known agonist or antagonist to a receptor for a non-GRP bombesin-like peptide; and
- c) a ligand binding fragment from a receptor for a bombesin-like peptide.

35. A method of treating a host having cancer or exhibiting abnormal expression of a receptor for a bombesin-like peptide, comprising administering to said host a therapeutically effective amount of a composition comprising:

- a) an antibody which binds to a receptor for a bombesin-like peptide;
- b) an agonist or antagonist to a receptor for a non-GRP bombesin-like peptide; or
- c) a ligand binding receptor, or fragment thereof, for a bombesin-like peptide.

36. A method of diagnosing for cancer in a host organism, comprising the steps of:

- a) contacting a sample from said host with a specific binding reagent to:
- i) a gene encoding a receptor for a bombesin-like peptide; or ii) a receptor for a bombesin-like peptide; and
- b) measuring the level of binding of said reagent to said sample.

37. A method of evaluating binding affinity of a test compound to a receptor for a bombesin-like peptide, said method comprising the steps of:

- a) contacting a sample containing said receptor with
- i) a labeled compound having a known affinity for said receptor; and; ii) said test compound; and

WO 92/16623 PCT/US92/02091

157

b) measuring the level of bound labeled compound, said amount being inversely proportional to the amount of test compound which bound to said receptor.

38. A kit for determining the amount of a receptor for a bombesin-like peptide in a sample, comprising a compartment with a labeled compound having a known binding affinity for said receptor.

39. A kit for assaying antibody against a receptor for a bombesin-like peptide in a sample, comprising compartments having a said receptor and an antibody detection means.

40. A compound known to modulate activity of a receptor for a bombesin-like peptide, selected by a method of:

- a) contacting said compound with isolated or recombinant receptor, or fragment thereof, for a bombesin-like peptide; and
- b) evaluating the effect on biological activity by said contacting.

41. Isolated DNA encoding the gastrin releasing peptide receptor or fragment thereof encoding a biologically active gastrin releasing peptide receptor polypeptide.

42. Isolated DNA which encodes a biologically active protein having gastrin releasing

peptide receptor activity and which is capable of hybridizing with the DNA of SEQ ID NO: 1.

43. The DNA of Claim 42 wherein said protein has the amino acid sequence of SEQ ID NO: 2.

44. Isolated DNA encoding proteins which are homologous to the gastrin releasing peptide receptor, and said DNA being isolated using gastrin releasing peptide receptor cDNA as a probe.

WO 92/16623 PCT/US92/02091

158

45. The DNA sequence according to Claims 41, 42, or 44 characterized in that it further comprises the respective regulatory sequences in the 5' and 3' flanks.

46. A DNA sequence hybridizing to a DNA sequence according to Claims 41, 42, or 44 and containing mutations selected from the group consisting of nucleotide substitutions, nucleotide deletions, nucleotide insertions and inversions of nucleotide stretches and coding for a protein having gastrin releasing peptide receptor activity.

47. A recombinant DNA molecule characterized in that it comprises a DNA sequence according to Claims 41, 42, or 44.

48. A recombinant DNA molecule characterized in that it comprises a DNA sequence according to Claims 41, 42, or 44 that is operably linked to a genetic control element.

49. The recombinant DNA molecule of Claim 48, characterized in that said control element is selected from the group consisting of prokaryotic promoter systems and eucaryotic expression control systems.

50. The recombinant molecule of Claim 47 wherein said molecule is an expression vector for expressing eucaryotic cDNA coding for the gastrin releasing peptide receptor in a prokaryotic or eucaryotic host, said vector being compatible with said host and wherein the eucaryotic cDNA coding for the gastrin releasing peptide receptor is inserted into said vector such that growth of the host containing said vector expresses said cDNA.

51. A host characterized in that the recombinant DNA molecule according to Claim 47 has been introduced into said host, and which expresses the protein encoded by said DNA.

WO 92/16623 PCT/US92/02091

159

52. The host of Claim 51 which is selected from the group consisting of: prokaryotes including gram negative and gram positive organisms including *E. coli*; lower eucaryotes including yeasts; and higher eucaryotes including animal cells and mammalian cells including human.

53. A recombinant protein which is encoded by a DNA sequence according to Claim 47 and which is substantially free of protein or cellular contaminants, other than those derived from the recombinant host.

54. A pharmaceutical composition comprising the recombinant protein of Claim 53 and a conventional pharmaceutically acceptable carrier and/or diluent.
55. A vector comprising DNA encoding the gastrin releasing peptide receptor or a fragment thereof encoding a biologically active gastrin releasing peptide receptor polypeptide.
56. The vector of Claim 55 wherein said DNA is under the control of a viral promoter.
57. The vector of Claim 55 which further comprises DNA encoding a selection marker.
58. The vector of Claim 55 wherein said DNA encodes a predetermined, site-specific mutant gastrin releasing peptide receptor which has greater than about 50% amino acid homology with the gastrin releasing peptide receptor of SEQ ID NO: 2 and which exhibits biological activity in common with the gastrin releasing peptide receptor of SEQ ID NO: 2.
59. A cell from a multicellular organism transformed with the vector of Claim 55.

60. The cell of Claim 59 which is a mammalian cell.
WO 92/16623 PCT/US92/02091

160

61. A method comprising culturing the cell of Claim 59 in a nutrient medium, permitting the receptor to accumulate in the culture and recovering the receptor from the culture.
62. The method of Claim 61 wherein the receptor is recovered from the culture medium.
63. Antibodies having binding affinity to the recombinant gastrin releasing peptide receptor, or fragments thereof.
64. The antibodies of Claim 63 which are raised against the gastrin releasing peptide receptor, or fragments thereof.
65. The antibodies of Claims 63 or 64 wherein said receptor has the amino acid sequence of SEQ ID NO: 2.
66. The antibodies of Claim 65 wherein said fragments are selected from the group consisting of the following partial amino acid sequences: residues 1-39, inclusive; residues 64-77, inclusive; residues 98-115, inclusive; residues 138-157, inclusive; residues 176-209, inclusive; residues 236-266, inclusive; residues 288-300, inclusive; and residues 330-385, inclusive.
67. The antibodies of Claim 63 which are non-neutralizing antibodies.
68. The antibodies of Claim 63 which are neutralizing antibodies.
69. The antibodies of Claim 63 which are conjugated to toxins.
70. The antibodies of Claim 63 which are conjugated to radionuclides.

71. A kit for determining the concentration of gastrin releasing peptide receptor in a sample comprising a labeled
WO 92/16623 PCT/US92/02091

161 compound having known binding affinity for the gastrin releasing peptide receptor, recombinant gastrin releasing peptide receptor, and a means for separating bound from free labeled compound.

72. The kit of Claim 71 wherein said means for separating is a solid phase for immobilizing the gastrin releasing peptide receptor.

73. The kit of Claim 71 wherein said labeled compound is a ligand.

74. The kit of Claim 73 wherein said ligand is gastrin releasing peptide.

75. The kit of Claim 71 wherein said labeled ligand is an antibody.

76. The kit of Claim 72 wherein said solid phase contains a capture molecule.

77. The kit of Claim 76 wherein said capture molecule is an antibody to the gastrin releasing peptide receptor.

78. A kit for determining the binding affinity of a test compound to the gastrin releasing peptide receptor comprising a test compound, a labeled compound having known binding affinity for the gastrin releasing peptide receptor, recombinant gastrin releasing peptide receptor and a means for separating bound from free labeled compound.

79. The kit of Claim 78 wherein said means for separating is a solid phase for immobilizing the solubilized gastrin releasing peptide receptor.

80. The kit of Claim 78 wherein said labeled compound is a ligand.
WO 92/16623 PCT/US92/02091

162

81. The kit of Claim 80 wherein said ligand is gastrin releasing peptide.

82. The kit of Claim 78 wherein said labeled ligand is an antibody.

83. The kit of Claim 79 wherein said solid phase contains a capture molecule.

84. The kit of Claim 83 wherein said capture molecule is an antibody to the gastrin releasing peptide receptor.

85. A method of treating patients having a disease or disorder associated with abnormal expression or abnormal triggering of the gastrin releasing peptide receptor comprising administering antibodies having binding affinity to the recombinant gastrin releasing peptide receptor.

86. A method of treating patients having a disease or disorder associated with abnormal expression or abnormal triggering of the gastrin releasing peptide receptor comprising administering recombinant gastrin releasing peptide receptor, or fragments thereof.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.